

## Focus Review

# Three decades of Wnts: a personal perspective on how a scientific field developed

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*Wnt* genes and components of *Wnt* signalling pathways have been implicated in a wide spectrum of important biological phenomena, ranging from early organismal development to cell behaviours to several diseases, especially cancers. Emergence of the field of *Wnt* signalling can be largely traced back to the discovery of the first mammalian *Wnt* gene in 1982. In this essay, we mark the thirtieth anniversary of that discovery by describing some of the critical scientific developments that led to the flowering of this field of research.

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## Introduction: how we learn about what we know

Knowledge differs from the growth of knowledge. To learn the facts about a subject, such as *Wnt* genes or the *Wnt* signalling pathway, one can consult an encyclopaedia, a textbook, or a conventional review article. To understand how those facts were unearthed and assembled into coherent concepts, it is necessary to probe the history of a field—to learn about the sequence of events, the logical and illogical connections between those events, and the people who participated in them.

We have approached this essay with more attention to historical development than to a full repertoire of facts. While interesting experimental results about *Wnt* genes and their effects on cells and organisms continue to appear at an accelerating pace, we believe that there is a great deal to learn about the scientific enterprise more broadly by looking back on the unusual way in which the knowledge about those genes has grown over the past three decades. Some under-

standing of *Wnt* signalling is now required of those who aspire to succeed in many prominent fields of biology—including organismal development, cancer research, and stem-cell biology. There is also a sizeable subset of biologists who define themselves primarily as students of *Wnt* genes, while also aligning themselves with cancer, developmental, or stem-cell biologists or with communities devoted to the fruit fly, worm, amphibians, mouse, or *Homo sapiens*. This cannot be said of those working on many other genes or signalling pathways, raising the interesting questions of how and why scientists organize themselves in unusual ways and view their subjects through certain kinds of lenses.

We have composed this essay on the occasion of the thirtieth anniversary of the published report that announced our discovery of what proved to be the first mammalian *Wnt* gene (Nusse and Varmus, 1982). Taking that report as an arbitrary starting point in the history of this field, we have tried to highlight the most significant ways in which the field increased in knowledge, enlarged in scope, and grew in disciples. As we emphasize, some of these advances were logical and straightforward, others were technically difficult and protracted, and yet others were serendipitous and surprising. We also note more briefly how the shape of the field was determined by certain beneficial attitudes and behaviours that may be worthy of emulation.

## The 'pre-history': mouse models for breast cancer and cancer-causing retroviruses preceded knowledge of *Wnt* genes

All modern science is built on earlier science. Accordingly, the discovery that launched the intense study of *Wnt* genes 30 years ago depended on at least two earlier and closely related lines of enquiry: mouse models of cancer and oncogenic retroviruses.

It had been known since the 1930s that certain strains of laboratory mice are highly susceptible to breast cancer, and that the disease is usually transmitted from mothers to offspring mice through the milk (Bittner, 1936; Korteweg, 1936). Later, the tumour-inducing activity was purified from the milk (Lyons and Moore, 1962), and the milk-transmitted factor was shown to be a morphologically atypical retrovirus, called the Mouse Mammary Tumour Virus or MMTV.

Although the study of oncogenic retroviruses can be tracked to the first decade of the 20th century, the basis of their cancer-causing properties came into focus only in the century's second half. The first great advances came from tissue culture assays for viral growth and cell transforming capacities and from the biochemical and genetic analysis of the RNA genomes of retroviruses isolated from chickens, mice, rats, and other experimental animals. These methods

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led to the discovery of distinct viral oncogenes, such as Src, Myc, and Ras, and their cellular precursors, called proto-oncogenes, a term denoting any cellular genes that could be converted to active cancer-causing genes (Bishop and Varmus, 1985). Conversion to oncogenicity could occur by the mechanisms that produced highly oncogenic retroviruses or, as was shown in time and described later in this essay, by a variety of other mechanisms, most commonly somatic mutations of several types.

The proto-oncogenes initially discovered by tracing viral oncogenes to their cellular origins were generally not related to each other, but they shared several properties. They had been conserved during evolution and were converted to cancer-causing genes by gain-of-function mutations—as first shown for retroviral oncogenes and later for activated cellular oncogenes found in human cancers. The rapid onset of cancer caused by many retroviruses reflected the ability of active viral oncogenes to transform many infected cells (Bishop and Varmus, 1985).

Before RN came to UCSF in 1980 to work with HV, we had both been interested in breast cancer in the mouse and in the general properties of retroviruses isolated from animals. Such viruses often cause haematological cancers and sarcomas, relatively infrequent types of cancers in human beings; only rarely do they induce epithelial carcinomas, the most common human cancers. So, we were curious about the mechanism by which MMTV might cause carcinoma of the breast. Unlike the most intensively studied cancer-causing retroviruses, however, there was no readily identifiable oncogene in the viral genome. But, like other retroviruses, MMTV was known to insert a DNA copy of its RNA genome into the host cell genome during infection. Moreover, MMTV caused cancer slowly, over the course of several months, unlike oncogene-containing retroviruses, suggesting that viral infection alone, while required for tumour induction, was not sufficient to transform a host cell—a property that had also been recognized in the study of certain leukaemia-inducing retroviruses found in birds and mice (Teich *et al*, 1982).

It was known, however, that tumours induced by such viruses were composed of cell clones defined by shared proviral integration sites (Cohen *et al*, 1979; Payne *et al*, 1981) implying that tumours consisted of the descendants of a single infected cell and were thus the outcome of rare events. The pattern of clonality raised the possibility that one of many infected cells had randomly acquired a provirus that could initiate tumorigenesis. For instance, the insertion of viral DNA might cause a mutation of a gene in the vicinity of the integration site, and the change might confer a growth advantage to that cell. While it was possible that such mutations caused loss-of-function mutations, by disrupting a host cell gene, an alternative and more attractive model was that a host cell gene was transcriptionally activated by the incoming provirus. The latter model was inspired by findings in yeast and prokaryotes, where transposable elements, which are often functionally and structurally similar to retroviral proviruses (Brown and Varmus, 1989), were known to activate residing host cell genes (Errede *et al*, 1980).

The model of gene activation by proviral insertion gained momentum when Bill Hayward's group showed that B-cell lymphomas caused by the slowly oncogenic Avian Leukosis Virus (ALV) commonly contained proviruses inserted near

the *c-myc* gene and that *c-myc* was overexpressed in those tumours (Hayward *et al*, 1981). Initially, activation of *c-myc* appeared to be the consequence of a transcriptional promoter present in an ALV provirus inserted upstream of the protein-coding exons of *c-myc* in the same transcriptional orientation (Hayward *et al*, 1981), but research in HV's group showed that proviruses could also enhance *c-myc* expression from the endogenous *c-myc* promoter when inserted downstream of the gene or upstream in either orientation (Payne *et al*, 1982).

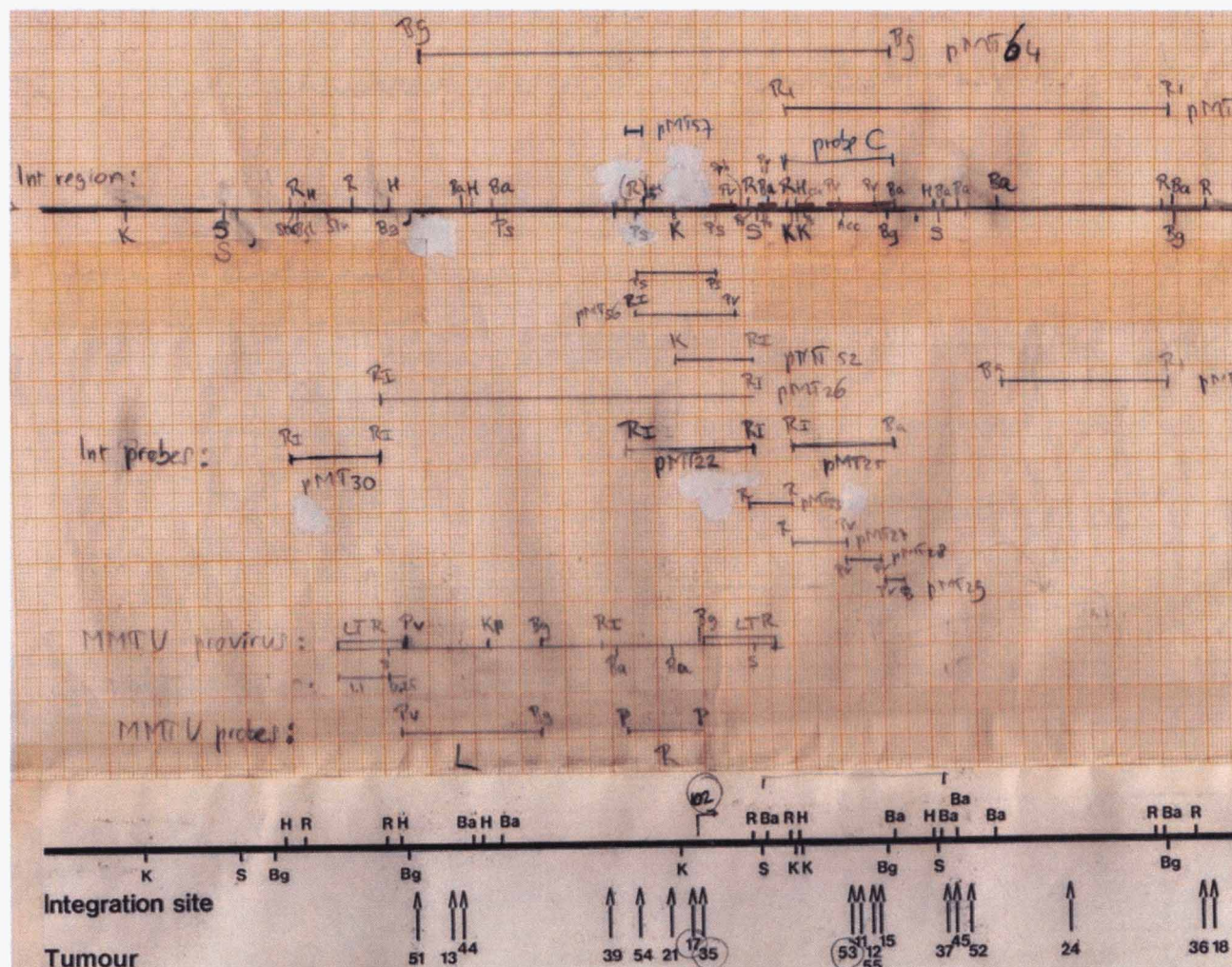
The discoveries concerning *c-myc* and ALV were of critical importance in cancer research, as it was the first time that a cellular homologue of a viral oncogene was shown to be activated in cancer by mutation. Soon thereafter, many kinds of mutations other than viral insertion mutations—gene amplifications, chromosomal translocations, and point mutations—were shown to activate *c-myc* and many other progenitors of retroviral oncogenes, in human as well as in animal tumours, without the intervention of retroviruses (Weinberg, 1983). The results with ALV and *c-myc* also set the stage for looking for a similar mechanism in tumours caused by other retroviruses, such as MMTV, with the prospect of finding proto-oncogenes that are not related to known viral oncogenes.

### Molecular cloning of *int1*, the first novel proto-oncogene identified by proviral tagging

To seek host cell genes that are activated by insertions of MMTV proviruses, we decided to do a systematic screen rather than to look for activation of only those proto-oncogenes known at the time, such as *c-myc*. We assumed, perhaps naively, that only one or perhaps a few genes in the mouse genome would confer tumourigenic growth when activated by MMTV in mammary cancer. This assumption implied that by comparing MMTV proviruses in multiple different tumours we would find common integration sites, or at least common regions, near those unusual genes. This segment of the genome would represent a so-called 'common integration site.' But the commonality would not reflect a predisposition to integrate MMTV at preferred locations in the mouse genome—all data, then and now, suggest that retroviral integration occurs quasi-randomly in host genomes—but instead would result from selection of cells that acquired a growth advantage when a provirus activated a nearby proto-oncogene.

The goal then became to isolate an integrated MMTV provirus plus its adjacent host cell DNA from a tumour by molecular cloning. We reasoned that we should start from a tumour with just one MMTV insertion. Most tumours in the strain we were using (C3H) carried multiple newly acquired proviruses, in addition to a few copies of inherited 'endogenous' MMTV DNA that were invariant among the arising tumours because they were products of ancient infections of the mouse germ line (Bentvelzen *et al*, 1970; Cohen and Varmus, 1979). Since it was difficult to know which of the multiple new proviruses would be functionally important, we looked for tumours carrying only one new provirus; this single insert should, according to the model, be near the relevant gene. Restriction fragments of host DNA derived from the vicinity of the provirus from that tumour would





**Figure 1** A working map of the mouse *int1* locus as drawn by RN and used from 1982 to 1984, with the position of various cloned genomic restriction fragments. Red lines indicate the presence of *int1* exons, mapped in 1984. Note the location of Probe C, a genomic fragment hybridizing with *int1* mRNA in mouse mammary tumours. At the bottom, the position of MMTV proviruses mapped in different tumours, with the position of the provirus in tumour #18, the starting point of the cloning of the locus, indicated at the right hand end.

then be used as probes for two purposes: to screen other tumours for integrations in the same domain and to measure transcription of local genes, both in tumours and in normal mammary tissue. Of course, today, with complete sequences of normal mouse genomes and powerful methods for sequencing tumour DNA in hand, precise mapping of insertion sites has become a relatively simple exercise. But at the time, these tools were not available, so a more cumbersome method was required.

We initiated the screen in October 1980, gathering over 30 MMTV-infected C3H mice with mammary tumours, and we found just one tumour (#18) with a single new provirus. Happily, that single tumour proved to suffice for our purposes. From the genome of this tumour, we cloned a 'junctional' fragment, containing a part of an MMTV provirus joined to adjacent chromosomal DNA, then derived smaller fragments containing only host cell DNA. With those fragments as probes, we 'walked' along the chromosome in either direction from the original proviral insertion site to obtain more probes for a broader region of the mouse genome (Figure 1). These were then used to examine DNA from other tumours for disruptions caused by MMTV proviruses. Indeed, many tumours in our collection had evidence

for MMTV DNA insertions in the region occupied by the single provirus in tumour #18; these were manifested by novel restriction fragments that hybridized with both MMTV and host cell probes. We assembled a map of the relevant region of the mouse genome, with the various proviral insertions in different tumours scattered throughout a region spanning  $\approx 30$  KB (Figure 1). It turned out that the original insertion site we cloned from tumour #18 was at one end of the cluster.

But where was the cellular gene that, according to our hypothesis, should have been activated as a result of these integrations? One of the probes (probe C, Figure 1) proved to be particularly helpful. It picked up integration events in many individual tumours and mapped approximately to the middle of the cluster of integrations. More importantly, the same probe also detected a tumour-specific mRNA—that is, a species not found in normal mammary gland tissue—on northern blots. This was the evidence we were looking for—a host gene, a putative proto-oncogene, had been transcriptionally activated by MMTV proviruses in multiple, independent tumours (Nusse and Varmus, 1982). We called the gene *int1* (to denote the first common integration site) and promptly submitted a manuscript that was published in



the journal *Cell* in November 1982 (Nusse and Varmus, 1982). The method we used, called proviral tagging, is now widely used to discover proto-oncogenes in cancers induced in mice by retroviruses or transposable elements and in cancers whose growth is accelerated in transgenic animals by these elements (Kool and Berns, 2009; Copeland and Jenkins, 2010).

The paper describing *int1* was well received, but its impact at that time was overshadowed by an avalanche of incredibly exciting developments in cancer research. In the same year, 1982, human cancers were found to have mutations in cellular RAS genes, and other human tumours were shown to contain chromosome translocations directly affecting *c-myc* (Bishop, 1983). Soon thereafter, equally explosive findings were announced, identifying oncogenic proteins as known factors governing growth control: the *erbB* oncogene was discovered to be derived from the EGF receptor gene, and both encoded protein-tyrosine kinases; and the precursor to the *v-sis* oncogene was shown to be the gene encoding PDGF, a secreted growth factor (Bishop and Varmus, 1985).

### The difficulties of defining the mechanism of action of the *int1* gene and its encoded protein

In the midst of all of the excitement about retroviral oncogenes and their progenitors, *int1* attracted relatively little attention beyond the confines of our laboratories, despite the many open questions that seemed important and interesting to us. Initially, in the absence of a nucleotide sequence, we had no clue how the gene would function. Work done in both our laboratories (HV at UCSF; and RN after his return to the Netherlands Cancer Institute in Amsterdam in 1982) elucidated the structure and sequence of the *int1* gene (van Ooyen and Nusse, 1984) and its cDNA (Fung *et al*, 1985), revealing no homology with any other gene or protein known at the time. We did notice, however, that the predicted protein sequence started with a signal sequence, indicating that the *int1* protein would be secreted. This opened up the exciting possibility that this protein might be an extracellular growth factor. But direct proof of this prediction turned out to be very hard to obtain. For many years, no one was able to produce or isolate significant quantities of the *int1* protein, a problem that was not solved until 2003 (see below). To make matters worse, generating useful antibodies to *int1* was an equally frustrating enterprise; in fact, detecting *int1* protein in cells or tissues remains an elusive goal even today. These problems, in particular the lack of active protein for experimental use, precluded conventional signalling assays in cell culture. As a result, indirect assays, such as those dependent on gene transfer, had to be used to study signalling events in cells expressing *int1*. In particular, the identification of specific *int1* cell surface receptors by binding assays was not possible until much later.

On the other hand, we did establish that expression of the *int1* gene could affect cell behaviour in a fashion that resembled conventional transformation and provided a biological assay: various mammary epithelial cell lines could be morphologically altered by overexpression of *int1*, albeit in a subtle way and rarely leading to formation of cells capable of growing into a tumour (Brown *et al*, 1986; Rijsewijk *et al*,

1987b). More dramatically, Ann Tsukamoto in HV's laboratory was able to recapitulate the oncogenic effect of *int1* in mice without resorting to virus infection: mice expressing an *int1* transgene under the influence of an MMTV transcriptional regulator developed cancer in the mammary gland within about 6 months of age (Tsukamoto *et al*, 1988). This established that *int1* is a *bona fide* proto-oncogene. These mice—and several others expressing *int1* under the control of inducible promoters—have since become widely used mouse models for studying breast carcinogenesis and for finding genes that can cooperate with the *int1* transgene during oncogenesis (MacArthur *et al*, 1995).

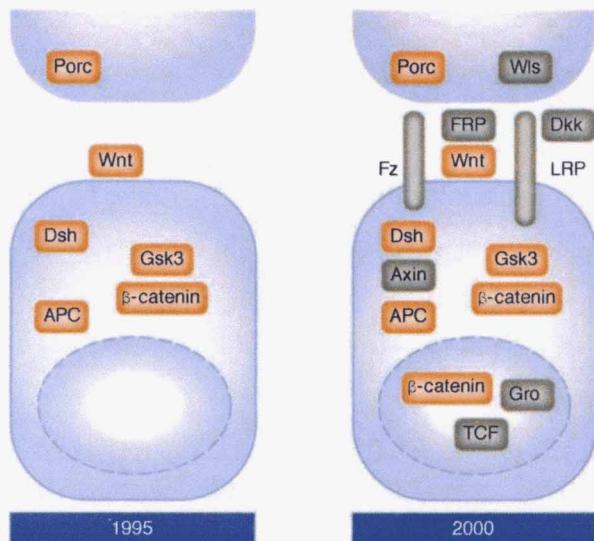
### Developmental genetics helped to reveal the function of *int1* when the gene was discovered to be the homologue of the *Drosophila* segment polarity gene, *Wingless*

How could the function of *int1* protein and the components of its signalling pathway be deciphered without a direct, convenient biochemical or cell-based assay? Fortunately, *int1* did have one advantage: a high degree of conservation across species. The human *int1* protein sequence turned out to be almost completely (99%) identical to that of the mouse homologue (Van Ooyen *et al*, 1985). Moreover, *int1*-related sequences appeared to be present in the DNA of *Drosophila melanogaster* as judged from molecular hybridization (Nusse *et al*, 1984).

At the time, the new molecular methods that were responsible for unveiling the genes central to cancer research had also re-energized efforts to understand embryogenesis, using the rich treasury of *Drosophila* developmental mutants. During the 1970s, genetic screens in *Drosophila* had unveiled a set of genes that were essential for the development of the embryo. Nüsslein-Volhard and Wieschaus (1980) showed specific patterning defects, ranging from abnormal segment numbers to polarity changes, in mutants for many of these genes. They coined the term 'segment polarity genes' for one class of mutants that shared a similar patterning phenotype during embryogenesis. One of the genes in this group was called *Wingless*; others, *Armadillo* and *Arrow*. The *Wingless* gene had actually been identified earlier as a weak mutant allele leading to loss of wing tissue, hence the name *Wingless* (Sharma and Chopra, 1976). Subsequent to the genetic screens, many of the segmentation genes were molecularly cloned, generating a treasure trove of reagents to study developmental mechanisms. For example, the expression patterns of these genes often produced stripes corresponding to body segments. By examining the expression pattern of one gene in the background of mutations in other genes, hierarchies of genetic interactions were uncovered, providing unparalleled insights into how embryos develop (Ingham, 1988).

RN and his colleagues cloned the *Drosophila* *int1* homologue and used polytene chromosome mapping (the genomics technology at the time) to locate the gene. It turned out to map close to *Wingless*, one of the segment polarity genes; a striped expression pattern observed with a *Drosophila* *int1* probe also suggested a role in segmentation (Rijsewijk *et al*,





**Figure 2** Wnt signalling components as known in 1995 and 2000.

1987a). Around the same time, Baker (1987) had cloned the *Wingless* gene by a P-element transposon tagging, a method akin to the proviral tagging methods we had used for *int1*. The gene he cloned had restriction maps matching our *Drosophila int1* clone. The genes were identical; the *int1* homologue in *Drosophila* was *Wingless*, one of the first examples of a gene involved in development and also activated in cancer (Rijsewijk *et al*, 1987a). This was an exciting discovery in its own right. In addition, the membership of *Wingless* in the segment polarity group promised to open doors to discovering the mechanisms of action of *int1/Wingless*, since it seemed likely that other genes in the group would interact with *int1/Wingless* genetically and biochemically. As we now understand, the core of its signalling pathway is indeed based on genetic relationships between segment polarity genes, with a key role for *Armadillo* (see below) (Figure 2).

It had long been argued by some that cancer can be considered akin to a developmental abnormality, a disease caused by cells that have escaped from the normal developmental constraints on proliferation and differentiation (Boveri, 2008). Implicated in cancer as well as in embryogenesis, *int1* became a poster child for these connections. Interest in the roles of *int1* in development was further strengthened by reports by McMahon and Moon (1989) showing that *int1* was implicated in embryonic axis formation in another animal, *Xenopus laevis*. *Xenopus* had been used classically to study morphological changes in development; more recently, injection methods had been used to perturb development by introduction of wild-type and mutant genes and proteins into early stage *Xenopus* embryos. McMahon and Moon (1989) found that ectopic expression of *int1* duplicated the dorsal axis, suggesting a role for *int1* as an organizer and an embryonic ‘inducer’, a signal between germ layers that would lead to pattern formation. Because of the speed and clarity of the embryological assays, *Xenopus* embryology became a mainstay of research on *int1* and other developmental signals.

In other organisms, *int1* expression patterns in embryos and other tissues suggested a diversity of functions in the development of tissues. For instance, *int1* was initially found to be expressed mainly near the midbrain and cerebellum at the mid-stage of mouse development and in a specific stage of male germ cell maturation (Shackleford and Varmus, 1987; Wilkinson *et al*, 1987).

## Int1 was one of the first genes to be knocked out by homologous gene targeting in mice, producing a developmental phenotype

During the late 1980s, Mario Capecchi and Oliver Smithies developed the groundbreaking technique for generating gene-specific mouse mutants by homologous recombination. After learning about the highly restricted pattern of expression of *int1*, Capecchi’s group chose the gene as one of its initial targets for application of this amazing technology. After breeding to homozygosity, disruption of *int1* produced a dramatically diminished cerebellum, accompanied by severe ataxia (Thomas and Capecchi, 1990). In McMahon’s laboratory, *int1* null mutants caused an embryonic lethal phenotype in the mid-brain also affecting the development of the cerebellum (McMahon and Bradley, 1990). Soon thereafter, it was also shown that a classical mouse mutation, *Swaying* (Lane, 1967), was an allele of the *int1* gene; when *int1* was cloned from *Swaying* mice, it proved to have a frameshift mutation (Thomas *et al*, 1991).

These several discoveries about *int1* mutant organisms in flies and mice were very striking. In a review article published in 1992 (Nusse and Varmus, 1992), we wrote: ‘With the benefit of hindsight, we now recognize that phenomena studied for several decades are the consequences of *Wnt* gene mutations. Viral insertion mutations regularly promote mammary tumours in laboratory mice (Bittner, 1936; Korteweg, 1936), a spontaneous frameshift mutation of mice (*swaying*) impairs cerebellar structure and function (Lane, 1967; Thomas *et al*, 1991) and *wingless* mutations in *Drosophila* can transform a wing to a notum or disrupt segment polarity (Sharma and Chopra, 1976; Nüsslein-Volhard and Wieschaus, 1980)’.

## Renaming *int1* as *Wnt1* and recognition of a *Wnt* gene family

Around 1990, it became clear that the *int* nomenclature had become inadequate and confusing. For example, additional screens for MMTV proviral insertion sites in tumours had yielded other activated genes, called *int2*, *int3*, and *int4* (Dickson *et al*, 1984; Gallahan and Callahan, 1987; Roelink *et al*, 1990). But by sequence comparisons, these genes were not usually related to *int1*. One MMTV target gene, initially called *int4*, did prove to be related to *int1* (Roelink *et al*, 1990). But the frequently activated *int2* gene, first identified by Clive Dickson and Gordon Peters (Dickson *et al*, 1984), turned out to be a member of the FGF family (Dickson and Peters, 1987). Interestingly, FGF genes were also implicated in normal development at about this time, sometimes in coordination with *int1*-related genes, as in mesoderm formation (Kimelman *et al*, 1992). Moreover, *int1* and *int2*



are sometimes co-activated in MMTV-induced breast tumours (Peters *et al*, 1986). *int3* was shown to be related to Notch, another important developmental regulator (Gallahan and Callahan, 1997).

At the same time that the *int* gene nomenclature was becoming unworkable, various experiments, including PCR-based homology screens, had revealed a large family of genes related to *int1* (Gavin *et al*, 1990). It would have been confusing to christen all these genes with the term 'int', whether or not they had been activated by proviral integration. To avoid further confusion, all those working on *int1* and its relatives, including *Wingless*, consented to a new hybrid name 'Wnt' (for *Wingless*-related integration site) to denote genes belonging to the *int1*/*Wingless* family, with *int1*, now called *Wnt1*, as the founding member (Nusse *et al*, 1991). (In accord with other recognized relationships, *int2* is now called *FGF3*, the *int3* gene is *Notch4*, and *int4* is *Wnt3A*.)

## The Wnt family as a vantage point to study gene evolution and development in metazoans

With the complete sequences of the genomes of many multicellular animals in hand, we now realize that vertebrates contain a family of 19 Wnt-related genes; pairs of these genes can often be placed in subfamilies that are highly similar to each other, perhaps reflecting gene duplications relatively recently in evolution (Gavin *et al*, 1990). Each of these genes seems likely to have a specific role in development or other processes; they are generally expressed in different cells and at different times in maturation (Gavin *et al*, 1990). Many of the *Wnt* genes have now been deliberately mutated in the mouse, almost always leading to striking phenotypes, including limb polarity defects and sexual dimorphic abnormalities (Parr and McMahon, 1995, 1998).

Even representatives of the earliest branches of the animal kingdom, such as *Hydra* and *Nematostella*, have the same number of Wnt subfamilies as vertebrates (Kusserow *et al*, 2005). Readily recognized orthologues of specific *Wnt* genes—for example, *Wnt1*—have been found throughout the entire animal kingdom, often expressed in tantalizing patterns (Kusserow *et al*, 2005). As a result, evolutionary biologists speculate that the early amplification and diversification of the Wnt family were at the roots of the increased complexity of animal body plans (Sidow, 1992; Holstein, 2012). It appears from such findings that *Wnt* genes were probably present in genomes prior to the split of the animal kingdom into protostomes and deuterostomes, are therefore at least 600 M years old, and may have a universal role in setting up the primary axis of animals (Petersen and Reddien, 2009; Niehrs, 2010; Holstein, 2012). However, it is also clear that single-cell organisms do not contain *Wnt* genes, nor do plants.

In the midst of all of these genes and families, it remains striking that *Wnt1* is one of the key Wnt family members and may have been the primordial one. *Wnt1* is the true orthologue of *Wingless*, a gene in *Drosophila* with numerous functions in later development as well as early embryogenesis. While there are six other Wnts in *Drosophila*, the others each play a minor role compared with *Wingless*. Moreover, there

are very useful temperature-sensitive alleles of *Wingless* to study its numerous functions. As a result of these attributes, *Wingless* has been a rich source for understanding developmental processes. In other organisms that have multiple Wnts, *Planaria* in particular, the true *Wnt1* orthologue also has a special place because of the requirement for it in regeneration (Gurley *et al*, 2010). While we do not understand why *Wnt1* is the most frequently activated gene in MMTV-induced breast cancer, it should be noted that we now know that *Wnt1* is closely linked to another *Wnt* gene that is often insertionally activated, *Wnt10B* (Lee *et al*, 1995). Thus, some MMTV inserts may have activated both genes, providing a greater growth advantage.

## Unexpected findings reveal the importance of the Wnt pathway in human cancers

From the time that *Wnt1* was discovered as an initiating gene in mouse mammary carcinogenesis, it remained of great interest and importance to establish whether *Wnt* genes were involved in any human cancer. Early tests for aberrant expression of *Wnt* genes in human breast cancer gave ambiguous results, at best, and no *Wnt* genes appeared to be mutated in any kinds of human tumours by DNA rearrangements or (as more recently documented by next generation sequencing of whole exomes or whole genomes) by point mutations (<http://www.sanger.ac.uk/genetics/CGP/cosmic>).

But work from a different angle changed our perceptions about the role of *Wnt* genes in human cancer, demonstrating that downstream components of the Wnt signalling pathway, rather than the *Wnt* genes themselves at the upstream end of the pathway, were commonly altered in several types of human cancer. The first news came from the study of colon cancer, and it was dramatic.

Around 1990, significant advances had been made in positional cloning of inherited human disease genes, including genes predisposing to several types of cancer. Among the hereditary forms of human cancer, adenomatous polyposis coli (APC), a trait associated with multiple polyps in the colon, often leads to colon cancer at a relatively early age. The corresponding mutations—often non-sense or frameshift mutations that produce truncated proteins—were found in an enormous gene called *APC*, which was cloned from human chromosome 5 in 1991 (Grodin *et al*, 1991; Kinzler *et al*, 1991). Identifying the human *APC* gene led to the cloning of a mouse homologue, subsequently shown by Bill Dove's group to be mutated in a mouse strain called Min (Multiple intestinal neoplasia) (Su *et al*, 1993). Just as in human families, the cancer trait in the Min mouse is produced by an APC truncating mutation, inherited in an autosomal dominant manner. But despite the new genetic insights into intestinal cancer, the function of the large APC protein posed a biochemical mystery.

Soon thereafter, the groups of Paul Polakis, Bert Vogelstein, and Ken Kinzler established that APC interacted in cells with a protein called  $\beta$ -catenin (Rubinfeld *et al*, 1993; Su *et al*, 1993). At the time,  $\beta$ -catenin had just been characterized by Masatoshi Takeichi and Rolf Kemler as a protein binding to the cytoplasmic domain of the adhesion molecule E-cadherin (Ozawa *et al*, 1989; Takeichi, 1990). Intriguingly, Pierre McCreau and Barry Gumbiner had found that *β-catenin* gene was a vertebrate homologue of the segment polarity gene,



*Armadillo* (McCrea *et al*, 1991), while Peifer and Wieschaus (1990) had established similarity between Plakoglobin (a  $\beta$ -catenin-related adhesion complex member) and *Armadillo*. Together, these findings suggested that APC and  $\beta$ -catenin/*Armadillo* were involved in regulating adhesion between vertebrate cells. Given the role of *Armadillo* in segment polarity, a function shared with *Wingless*, a model emerged in which Wnt/*Wingless* signalling controlled cell adhesion in development (Peifer and Wieschaus, 1990; Peifer *et al*, 1993), and the same adhesion-based mechanism could control growth of cells in tissues and cause cancer when misregulated.

While this was tantalizing, there were also reports that  $\beta$ -catenin/*Armadillo* was present in the nucleus, as well as at the cell membrane (Funayama *et al*, 1995). Other publications mentioned that injection of antibodies to  $\beta$ -catenin/*Armadillo* could induce dorsal axis duplication in *Xenopus* (McCrea *et al*, 1993), possibly by stabilizing the  $\beta$ -catenin/*Armadillo* protein; yet others claimed that depletion of maternal  $\beta$ -catenin/*Armadillo* could eliminate the dorsal axis (Heasman *et al*, 1994). These observations would ultimately all make sense:  $\beta$ -catenin is a key participant of Wnt signalling, but the molecular mechanisms remained unexplained until a few years later.

In deciphering the cascade of events between the Wnt signal and the role of  $\beta$ -catenin/*Armadillo*, the genetic interactions between a protein kinase called Glycogen Synthase Kinase 3 (GSK3) and other Wnt components proved to be of critical importance. Norbert Perrimon and colleagues showed that GSK3 (the *Drosophila* homologue was called zeste-white 3, also known as shaggy) was a negative regulator of the pathway; at the genetic level, Wnt/*Wingless* acted as a GSK3 inhibitor (Siegfried *et al*, 1992). Until then, GSK3 was known for its role in glucose metabolism (Dent *et al*, 1989), so its newly discovered role in developmental signalling was certainly surprising.

### **A Wnt signalling cascade from the cell surface to the nucleus, an unusual pathway experimentally assembled from several different models**

By 1995, the combined results from fly and mouse genetics, *Xenopus* embryology, and fly and mammalian cell culture experiments had generated an outline of a Wnt signalling pathway (Figure 2). It became clear that Wnt signalling was unusual compared with the other pathways known at the time: those consisted mostly of successions of protein phosphorylations, with protein associations based on recognition of phosphorylated domains. In Wnt signalling, the most upstream known component was a cytoplasmic protein of uncertain biochemical function, Dishevelled, which then was proposed to inhibit the abundant GSK3 protein kinase (Peifer *et al*, 1991; Siegfried *et al*, 1992, 1994; Noordermeer *et al*, 1994). GSK3 was known to be a negative regulator of  $\beta$ -catenin/*Armadillo* and was found in a complex with  $\beta$ -catenin/*Armadillo*, together with the APC protein (Rubinfeld *et al*, 1996). The role of the GSK3 kinase activity as a suppressor of Wnt action was confirmed by injecting dominant-negative (kinase-dead) mutants of GSK3 into early *Xenopus* embryos; this maneuver produced a phenocopy of

the effect of Wnt1, duplication of the dorsal axis, that had been reported previously by Moon and MacMahon (Dominguez *et al*, 1995; He *et al*, 1995; Pierce and Kimelman, 1996).

A critical next step was to determine how phosphorylation by GSK3 governed  $\beta$ -catenin. This seemed likely to occur by control of the level of  $\beta$ -catenin protein. In cells activated by Wnt, levels of  $\beta$ -catenin are commonly increased (Riggelman *et al*, 1990; Peifer *et al*, 1994; Van Leeuwen *et al*, 1994) by stabilizing the  $\beta$ -catenin protein, not by an increase in its synthesis. Several highly conserved Ser/Thr phosphorylation sites near the amino terminus of  $\beta$ -catenin were proposed as possible targets for phosphorylation by GSK3 (Peifer *et al*, 1994). As shown by Rolf Kemler's and Randall Moon's groups, phosphorylated  $\beta$ -catenin is targeted for degradation by the ubiquitination/proteasome pathway (Yost *et al*, 1996; Aberle *et al*, 1997; Orford *et al*, 1997), with a critical role for F-box proteins (Jiang and Struhl, 1998). Eliminating one or more of the N-terminal phosphorylation sites stabilizes  $\beta$ -catenin, producing abundant protein highly active in *Xenopus* axis formation assays (Yost *et al*, 1996). As a result, there are striking parallels between the Wnt, Hedgehog, and NF- $\kappa$ B signalling pathways; in all three cases, regulated signalling depends on degradation of a key pathway component by the ubiquitination/proteasome pathway after phosphorylation (Jiang and Struhl, 1998; Maniatis, 1999).

The study of the molecular pathology of colon cancers then offered a remarkable example of the predictive power of knowledge about signalling. As mentioned earlier, inherited mutations in the APC gene were known to cause the familial disease APC, and somatic mutations in APC were found in most (ca. 85%) but not in all sporadic colorectal cancers. Why not all? Could mutations affecting other components of the Wnt signalling pathway substitute for APC mutations? Or did other mutant signalling pathways drive those tumours? Paul Polakis, Bert Vogelstein, Hans Clevers and their colleagues looked specifically for altered  $\beta$ -catenin genes in APC wild-type tumours, on the supposition that  $\beta$ -catenin protein could be stabilized by mutations affecting the N-terminus as well as by loss of APC. Indeed they found that about 5–10% of sporadic colon cancers had mutations, often short deletions, that removed or changed the phosphorylation sites that target  $\beta$ -catenin for degradation (Korinek *et al*, 1997; Morin *et al*, 1997; Rubinfeld *et al*, 1997). Subsequently, mutations have also been found in another component of the degradation complex, Axin, in colorectal and other types of cancers (Satoh *et al*, 2000).

This other component of the  $\beta$ -catenin/*Armadillo*/GSK3/APC complex has an interesting history. Cloned by Frank Costantini as a mouse developmental mutant called fused (Zeng *et al*, 1997), the Axin gene encodes a protein that shares homology with Dishevelled, suggesting possible participation in the Wnt pathway. This turned out to be the case. Axin is now known to participate in the  $\beta$ -catenin destruction complex, together with APC and GSK3 (Behrens *et al*, 1998; Ikeda *et al*, 1998). Using a novel cell-free system for studying  $\beta$ -catenin degradation, Marc Kirschner and colleagues showed that Axin is the rate-determining component of the complex, even though it was the most recently identified (Lee *et al*, 2003). Axin has a similar role in intact mammalian cells (Li *et al*, 2012).



After these several elements were implicated in Wnt signalling, two apparent and important gaps in the pathway remained, one at each end of the pathway (Figure 2). On the upstream end, there were no proteins known to recognize extracellular Wnt proteins and transmit a signal to the cell's interior (Wnt receptors). On the downstream end, the anticipated effects on gene expression through transcriptional control could not be explained because  $\beta$ -catenin does not have the expected physical attributes of a transcription factor, and no established transcription factor was known to partner with it. Then, in a single year, 1996, these gaps were closed, generating excitement in the growing Wnt field (Figure 2).

### Wnt receptor proteins were known in other contexts before their roles in Wnt signalling were uncovered

After many trial-and-error searches, one class of the elusive Wnt receptors was identified: the Frizzled transmembrane proteins. Originally found in mutant screens by Calvin Bridges (Bridges and Brehme, 1944), *Frizzled* had been identified in *Drosophila* as a gene required for planar polarity (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987), the orientation of cells in tissues. Paul Adler and colleagues showed Frizzled to encode a seven-pass transmembrane protein (Vinson *et al.*, 1989). Genetically, at least, Frizzled interacted with Dishevelled, which was shown by Perrimon and Mahowald (1987) to be involved in Wingless signalling as well. While this suggested that Frizzled could mediate Wingless signalling, the absence of an embryonic segment polarity phenotype in Frizzled mutants indicated otherwise.

Here serendipity stepped in. Jeremy Nathans and his colleagues found a Frizzled homologue among components of a human retinal cDNA library that had been made to pursue their interests in the molecular biology of vision. When the cDNA was used to seek homologues in a library of *Drosophila* DNA, a second *Drosophila* Frizzled gene (*Dfz2*) was cloned, and the *Dfz2* gene displayed a striped pattern of gene expression in the embryo, implying that it might be directly involved in segment polarity. A collaboration between the Nathans and RN laboratories revealed that the Wingless protein, which RN's laboratory had solubilized at the time, could bind to *Dfz2* and, more weakly, to Frizzled itself (Bhanot *et al.*, 1996). Moreover, in cultured *Drosophila* cells that did not express Frizzled genes, transfection of an expression vector containing Frizzled genes conferred active signalling, as demonstrated by an increase in Armadillo ( $\beta$ -catenin) levels (Bhanot *et al.*, 1996). Genetic and other interactions between Frizzleds and Wnts were also reported by the groups of Randall Moon and Robert Horvitz (Sawa *et al.*, 1996; Yang-Snyder *et al.*, 1996).

Just like Wnts, Frizzleds form a large gene family in all branches of metazoan animals. Genetically, *Frizzled* genes are often redundant and display phenotypes only when mutated in combination with other family members (Ye *et al.*, 2011). In *Drosophila*, this was shown for Frizzled and *Dfz2* using dsRNA interference technology (Kennerdell and Carthew, 1998). (Interestingly, this occurred in the same year that this revolutionizing method to inhibit gene

expression was first reported by Andy Fire and Craig Mello; Fire *et al.*, 1998). By using loss-of-function mutations in Frizzled and *Dfz2*, Eric Wieschaus, Gary Struhl, Ken Cadigan, and Krishna Bhat uncovered a segment polarity phenotype indistinguishable from phenotypes characteristic of the other genes in that class—but only as double mutants, explaining why these receptor genes were not in the original Nüsslein-Volhard/Wieschaus collection (Bhat, 1998; Bhanot *et al.*, 1999; Chen and Struhl, 1999; Muller *et al.*, 1999). Despite the evidence for redundancy, Frizzled proteins have different affinities for different Wnts (Rulifson *et al.*, 2000), indicating a high degree of specificity in their interactions. However, persistent experimental problems with the biochemistry of Wnt proteins have hampered systematic surveys of the interactions.

To complement the Wnt receptor story, the *Drosophila* gene *Arrow*, one of the last segment polarity genes to be identified, was cloned by Stephen DiNardo and colleagues a few years later. Arrow proved to be a member of the Low density lipoprotein receptor-Related Protein (LRP) family of receptors (Wehrli *et al.*, 2000). Based on additional genetic data from Bill Skarnes, who made LRP mouse mutants, and biochemical experiments from Xi He's laboratory, a model emerged in which Arrow/LRP is a co-receptor for Wnts, physically adjacent to Frizzleds in the cell membrane (Pinson *et al.*, 2000; Tamai *et al.*, 2000). When signalling to downstream components, however, Arrow/LRP may be the key player. Its cytoplasmic tail is phosphorylated as a consequence of Wnt binding and interacts directly with GSK3 and Axin (Mao *et al.*, 2001b; Tamai *et al.*, 2004; Davidson *et al.*, 2005; Zeng *et al.*, 2005) and Frizzled's intracellular role in signalling may be limited to binding Dishevelled (Macdonald *et al.*, 2009). Arrow/LRP is also the target of several Wnt antagonists including the protein Dickkopf, isolated by Christof Niehrs (Glinka *et al.*, 1998; Bafico *et al.*, 2001; Mao *et al.*, 2001a; Semenov *et al.*, 2001). The Dickkopf-Wnt antagonism is conserved across many animal phyla (Guder *et al.*, 2006), illustrating the ancient nature of Wnt signalling in animal development and evolution.

Eddy De Robertis, Jeremy Nathans, Jeff Rubin, and their co-workers have uncovered several other Wnt antagonists, in addition to Dickkopf. These are secreted molecules usually consisting of Wnt receptor domains that bind to Wnt itself. Some of these molecules have names such as FRP or FRZB, reflecting their similarity to the Frizzled receptor (Finch *et al.*, 1997; Leyns *et al.*, 1997; Rattner *et al.*, 1997). Others, such as the WIF protein (Hsieh *et al.*, 1999), are unrelated to Frizzled. These proteins are likely involved in fine-tuning the concentration of active Wnt outside cells.

The tandem arrays of Frizzled and LRP are not the only Wnt receptors, as there are various members of the trans-membrane tyrosine kinase family that serve to receive Wnts; these include the ROR (Oishi *et al.*, 2003; Mikels and Nusse, 2006) and Derailed/RYK (Yoshikawa *et al.*, 2003) proteins. Interestingly, these two classes of molecules have different Wnt binding modules: the RORs contain a CRD domain similar to the Frizzled CRD, while Derailed/RYK is related to the WIF protein mentioned above (Patthy, 2000). Wnt interactions with these receptors often lead to effects in cells that are unrelated to  $\beta$ -catenin, possibly mediating 'non-canonical Wnt signalling' (van Amerongen *et al.*, 2008).



## Another unexpected but previously well-known protein, TCF/LEF1, explains the role of $\beta$ -catenin in the Wnt signalling pathway

The TCF/Lef1 protein proved to be the long-sought Wnt transcription factor in the nucleus. Discovery of the critical interaction between this protein and  $\beta$ -catenin highlights one of the themes of this essay: historically, the map of Wnt signalling was assembled by merging evidence from several different cell types and organisms. TCF/Lef1, an HMG box-containing transcription factor, was first implicated in immune T-cell gene expression (Travis *et al*, 1991; van de Wetering *et al*, 1991; Waterman *et al*, 1991) without any evident link to Wnt signalling. Working separately on *C. elegans*, Jim Priess and colleagues identified an HMG-box family member, POP1, involved in mesoderm specification in the worm embryo (Lin *et al*, 1995), initially also without connections to the Wnt pathway.

Soon thereafter, a surprising discovery was reported: TCF/Lef1 could interact with  $\beta$ -catenin, considered at that time to be an adhesion molecule. Hans Clevers extended his earlier work on TCF/Lef1 to make this finding (Molenaar *et al*, 1996), while Walter Birchmeier (Behrens *et al*, 1996) and Rolf Kemler (Huber *et al*, 1996) started from  $\beta$ -catenin to establish binding to TCF/Lef1. Using *Drosophila*, Mariann Bienz and Rudi Grosschedl (Riese *et al*, 1997) found that Wingless signalling was mediated by TCF/Lef1 while Konrad Basler used mutagenesis screens to find a gene called *Pangolin* (Brunner *et al*, 1997), the single TCF/Lef1 homologue in *Drosophila*. Around the same time, continued investigations into *C. elegans* embryogenesis by Jim Priess, Craig Mello, and Bruce Bowerman unveiled that the set of MOM genes implicated in lineage choices were members of the Wnt pathway, including Wnt itself (MOM2), Porcupine (MOM1), and Frizzled (MOM5). All of these MOMs converged on POP1 as a transcription factor and WRM1 as a  $\beta$ -catenin-related gene (Rocheleau *et al*, 1997, 1999; Thorpe *et al*, 1997).

In many contexts, TCF/Lef1 can switch between two states. When bound to Groucho, it acts as a repressor of target genes; but when Groucho is displaced by  $\beta$ -catenin, the same target genes are transcriptionally activated (Cavallo *et al*, 1998; Daniels and Weis, 2005). Crystallographic studies by Bill Weis and Wenqing Xu revealed the molecular details of the binding between TCF,  $\beta$ -catenin, and other proteins. The structure of  $\beta$ -catenin contains a groove made by the 'Armadillo' repeats in the protein, explaining how  $\beta$ -catenin can interact with several different partners, including TCF/Lef1, E-cadherin, and APC (Huber *et al*, 1997; Graham *et al*, 2000).

These discoveries about the interaction between  $\beta$ -catenin and TCF/Lef1 were in more than one respect very significant, as they not only closed the gaps in the Wnt pathway but also provided unparalleled tools for experiments. TCF/Lef1 recognizes a well-defined DNA binding site. By multimerizing this sequence, Hans Clevers and colleagues generated very convenient luciferase-based Wnt reporters, called Top-FLASH and now widely used in the Wnt field to measure signalling (Korinek *et al*, 1997). There are now numerous genes known to have TCF/Lef1 binding sites in their promoters and hence likely to be transcriptional targets for the Wnt signalling pathway in at least some cell types; among these genes are several implicated in cancer, such as c-myc (He *et al*, 1998).

## At last, the purification of active Wnt protein

As we have recounted, by the end of the 20th century we had a blueprint of the Wnt signalling pathway and a readout for the pathway, both of which were missing in the two previous decades in which *Wnt* genes were intensively studied (Figure 2). Still lacking, however, was the purification of any active Wnt protein, a problem that we and many other researchers had been working on since the initial cloning of Wnt1. Why were Wnt proteins so much more refractory to biochemical purification than many other secretory proteins? Were they modified in a fashion that rendered them insoluble or highly adherent?

It was known from Norbert Perrimon's work and *Drosophila* genetics that export likely required a specialized protein encoded by Porcupine. Evidence that Porcupine encoded a putative acyltransferase (Kadowaki *et al*, 1996; Hofmann, 2000) suggested that detergents might be needed to keep lipid-modified Wnt proteins soluble during extraction from cells. With the help of assays that judged Wnt activity in extracts based on increased  $\beta$ -catenin levels in cells, Karl Willert in RN's laboratory finally managed to break through the purification barriers (Willert *et al*, 2003). (Coincidentally, Willert had obtained his PhD working with HV at UCSF.) Wnts were found to be indeed covalently attached to lipids, explaining to some extent their resistance to biochemical manipulation (Willert *et al*, 2003). It is now recognized that secretion of Wnt proteins is a complex process, involving a dedicated enzyme (Porcupine; Kadowaki *et al*, 1996) and secretory proteins that are specific for Wnt signals. Among these is the multiple-pass transmembrane protein Wntless/Evi, identified by the groups of Konrad Basler and Michael Boutros, once again using the *Drosophila* genetic resources that have over the years been so instrumental in understanding the details of Wnt signalling (Banziger *et al*, 2006; Bartscherer *et al*, 2006; Port and Basler, 2010).

## A growing and very active field of Wnt signalling

The availability of Wnt proteins and more quantitative reporters (e.g., TopFlash) as reliable end points for signalling have simplified the study of Wnt signalling in cell culture, attracting many new investigators. The generation of Wnt reporter mice, initially Top-gal animals from Elaine Fuchs (DasGupta and Fuchs, 1999) and later animals with transgenic markers driven by Axin 2 promoters (Lustig *et al*, 2002; van Amerongen *et al*, 2012), provided yet more experimental opportunities. One can now trace Wnt-responding cells in any tissue of the mouse, examine the origin of these cells, and follow their fate in normal settings or after injury (Barker *et al*, 2007, 2010; van Amerongen *et al*, 2012). These new experimental tools have led to a rapidly growing list of Wnt signalling components, built on the core pathway.

Wnt signalling is clearly complicated and unusual when compared with other growth factor cascades. At various nodes in the Wnt pathway, there are links to cyto-architectural proteins, such as those involved in adhesion and cell polarity (Nelson and Nusse, 2004). The Wnt pathway is clearly important for cell fate changes and the control of





**Figure 3** Participants of the 1991 Wnt meeting at UCSF. From left: RN, Andrew McMahon, Arend Sidow, Vladimir Pecenka, John Mason, Lee Fradkin, HV, Henk Roelink, Jasprina Noordermeer, Supriya Shivakumar, Frank van Leeuwen, Cindy Harryman, Jean-Paul Vincent, Jackie Papkoff, two unidentified people, Tony Brown, a third unidentified person, Helen Kwan. Top row, from left: Karl Willert, Neil Parkin, and Jan Kitajewski.

gene expression, but Wnt signalling can also influence how cells are shaped and polarized and how they divide (Veeman *et al.*, 2003). Hitoshi Sawa, Bruce Bowerman, and Craig Mello have provided conclusive evidence for a major role for Wnt signalling in asymmetric cell division in *C. elegans* and the annelid worm *Platynereis dumerilii* (Rocheleau *et al.*, 1999; Schneider and Bowerman, 2007; Sugioka *et al.*, 2011). Given the multiple roles of the Wnt pathway in development, these cell biological phenotypes are perhaps not surprising and have opened fertile ground for further research.

### **Sociology of the field of Wnt signalling: annual meetings, sharing information, and a dedicated website**

A history of Wnt signalling would be incomplete without a few comments on the sociology of the field, which, we believe, has several unusual aspects. Those features have contributed to one of the overarching characteristics of this field: the propensity of investigators working on *Wnt* genes and Wnt signalling to identify themselves, at least in part and often primarily, as students of Wnts, regardless of whether they are cancer biologists, developmental biologists, or biochemists. First and foremost among the unifying activities are the annual (or sometimes bi-annual) Wnt meetings. These are organized by working scientists in the field, not by institutions or meetings specialists, in a very informal, low-cost, but effective way. No one, except a keynote speaker, receives an invitation accompanied by a promise of reimbursements; all others are expected to get there, find food and lodging, and arrange to cover expenses. Nevertheless, the meetings are well attended by many principal investigators, not by just trainees. The meetings started in on a small scale in 1990 as regular gatherings of our two nearby laboratories, after RN had moved to Stanford from Amsterdam. We then

asked members of other laboratories to attend as well; Figure 3 shows most of the attendees at the 1991 meeting at UCSF. After HV moved to the NIH in 1993, we continued the gatherings; attendance soon grew to B 300 people, as our own trainees started their own laboratories to work on *Wnt* genes, and others joined the field. Many of the discoveries we have presented in this essay were first made public during Wnt meetings, including the identification of Frizzleds as Wnt receptors and TCFs/Lefs as transcription factors in Wnt signalling, both at the 1996 meeting at Stanford (Figure 4); and the Arrow/LRP findings were first reported at another Wnt meeting, at Stanford in 1999. The meetings have covered an increasingly wide range of subjects and biological systems related to Wnt signalling, and their popularity attests to the loyalty of Wnt researchers to the subject matter in its many manifestations.

Wnt meetings have also helped to establish a culture of sharing information and reagents. An example of the congenial relationship was the unanimous and friction-free acceptance of the new nomenclature (from *int* to *Wnt*) when it was felt that this would benefit the field and the general comprehension of its work. In parallel to the informal Wnt meetings in the United States, there have been numerous other Wnt conferences throughout the world, perhaps a bit more official but still in the same spirit of open exchange and camaraderie.

In 1996, around the time that the Internet became easier to navigate with browsers such as Netscape, RN started and still maintains a website, the Wnt homepage (<http://wnt.stanford.edu>). The various pages list genes and signalling diagrams, in many cases linked to other genome databases. But the site is also used to announce meetings and to supply information on Wnt technology and reagents, and has become a popular resource for the world-wide Wnt community and for others seeking information about *Wnt* genes, as appreciation of their importance has expanded.





**Figure 4** Celebration of discovery of Frizzleds as Wnt receptors at the 1996 Wnt meeting at Stanford. From left: Jeremy Nathans, Matthew Scott, RN, and HV.

### Contemporary Wnt signalling systems, including stem cells, and an outlook

It is now clear that Wnt signalling is widely implicated in diverse biological processes. For instance, the large majority of developmental decisions that cells make during embryogenesis and thereafter appears to be coordinated, in large part, by Wnts. Scientists are beginning to understand how organs grow and regenerate after injury, and it is clear that Wnt signalling has major functions in these processes as well. A particularly prominent example of the centrality of Wnt signalling is the recently recognized role of Wnts in maintaining stem cells. The choices that stem cells make to self-renew or to differentiate are very much dependent on extrinsic signalling factors coming from a niche. Wnt signals are widely active as niche factors, as illustrated by the identification of the LGR5 receptor as a Wnt target gene in many different kinds of adult stem cells (Barker *et al*, 2007) and the requirement for TCF4 to maintain stem cells in the intestine (Korinek *et al*, 1998).

The discoveries related to stem cells have also illuminated the connections between Wnt and cancer: in a simple but likely correct view, stem cells are normally dependent on external Wnts for self-renewal, but when a negative Wnt regulator such as APC is mutated in stem cells, cells with markers of early lineage development proliferate in an uncontrolled manner, producing cancers of the colon and other organs. In the mammary gland, where we first identified Wnt1 as an oncogene, stem cells are also Wnt dependent (Shackleton *et al*, 2006; Zeng and Nusse, 2010; van Amerongen *et al*, 2012), and Wnt1-induced tumours bear hallmarks of normal mammary stem cells (Li *et al*, 2003).

Wnt signalling mutations have also been implicated in a growing list of degenerative diseases. Important among these are bone density abnormalities with dysfunctional LRP receptors (Gong *et al*, 2001) and retinal degeneration with Frizzled mutations (Robitaille *et al*, 2002). Some metabolic disorders, including diabetes mellitus, have been associated with alterations in Wnt pathway genes (Grant *et al*, 2006).

It has been gratifying to witness growth of the Wnt field, from the finding of a single cancer gene in a mouse model to a

rich system branching out to influence so many aspects of metazoan biology and human disease. While outsiders may be intimidated by the current size of the field and the biochemical complexities of Wnt signalling, we suggest that there are still many fundamental aspects of Wnt-related biology to be discovered, understood, and exploited. Increasingly, structures of Wnt signalling components are being elucidated, often in complexes with their partners (Dann *et al*, 2001; Schwarz-Romond *et al*, 2007; Ahn *et al*, 2011; Chen *et al*, 2011; Cheng *et al*, 2011); but important aspects of the pathway's molecular machinery and biochemical regulators remain incompletely defined.

Progress in the Wnt field is much more rapid today than it was in the early history of this field, thanks to more sophisticated tools: Wnt-specific assays and materials and more general methods in structural biology, genetics, and cell biology. For instance, recognition of the role of Wnt signalling in stem-cell regulation has already led to the use of Wnt proteins or Wnt agonists to expand stem cells in culture (Sato *et al*, 2010; Zeng and Nusse, 2010; ten Berge *et al*, 2011). On the other hand, despite the evidence for widespread involvement of Wnt signaling in human carcinogenesis, the kinds of targeted cancer therapies that are now being developed against components of several other signaling pathways have not yet been produced to interfere with the Wnt pathway. Among the most significant challenges in future research in the Wnt field is the identification of effective and specific Wnt pathway inhibitors for use in cancer and other diseases. We expect that further understanding of the intricacies and varieties of Wnt signaling will help to achieve these important goals.

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### Conflict of interest

The authors declare that they have no conflict of interest.



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